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Effects of antibiotics on control of angular leaf spot of cotton

Paul Franklin Street
Iowa State College

EFFECTS OF ANTIBIOTICS ON CONTROL OF ANGULAR LEAF SPOT OF COTTON

by

Paul Franklin Street

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Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

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INTRODUCTION

The disease of cotton known as angular leaf spot and bacterial blight has been recognized as one of the three most important diseases of this valuable crop (Chester, 1950). It was first discovered by Atkinson in 1891 and the causal agent was proven to be Xanthomonas malvacearum (E.F.S.) Dow. by Erwin F. Smith in 1901. The bacteria are seed bourne (Edgerton, 1912a) and infect the cotyledon during germination (Tennyson, 1936), causing water-soaked lesions which produce an exudate. The bacteria from the exudate are disseminated by wind blown rain (Faulwetter, 1919) to surrounding plants where entrance is gained through stomates (Figure 1a). Leaf tissues are affected first between the veins and the lesions assume a shape which gives rise to the name "angular leaf spot" (Figure 1b). When the bacteria enter petioles or stems dark brown lesions develop which are described as the "black arm" phase (Edgerton, 1912a). Infection of the boll results in depressed water-soaked lesions which develop into the "boll rot" phase (Edgerton, 1912a).

Losses due to this disease are difficult to determine since the host plants will usually have two and sometimes all three of the disease phases at one time. Disease appraisal methods should include losses from all three forms of the disease (Chester, 1950).

Two avenues of approach are available for studies on the control of this disease: (1) Breeding for resistance (Bird, 1954), and (2) Using chemicals as protectant and eradicants. The present study is concerned with the second approach.

Control of wildfire, a bacterial disease of tobacco, with foliar

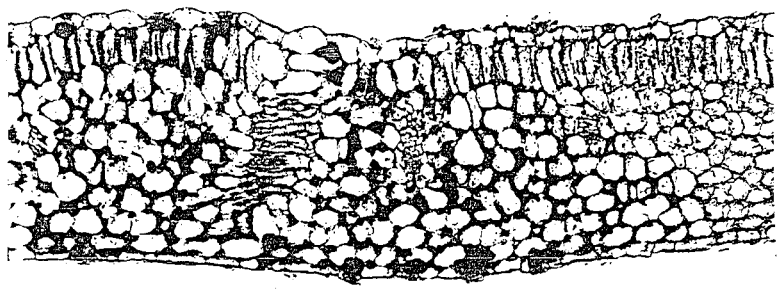

Figure 1a. Bacteria in substomatal spaces of cotton cotyledon (Photograph by Dr. J. E. Sass)

Figure 1b. Lesions on mature cotton leaf typical of angular leaf spot

applications of 200 ppm. of the antibiotic, Streptomycin, (Heggested and Clayton, 1954) suggested similar results might be obtained if a group of antibiotics were applied to cotton plants exposed to infection by the angular leaf spot bacterium. Experiments I to IV reported herein are attempts to carry out this suggestion.

Control of numerous plant diseases has been achieved with chemicals applied to the seeds prior to planting. Since angular leaf spot of cotton is caused by a seed bourne bacterium, the present study is concerned with attempts (Experiments V and VI) to find chemicals effective on seeds as protectants for increasing stands of seedlings and in seeds as eradicants for decreasing the amount of cotyledonary infection from the bacteria inside the seeds.

REVIEW OF PERTINENT LITERATURE

Bacterial blight of cotton caused by Xanthomonas malvacearum (E.F.S.) Dawson was first reported by Atkinson (1891). Water soaked lesions, typical of bacterial diseases, develop between veins and veinlets of leaves giving rise to the common name, angular leaf spot. That the lesions are teeming with bacteria was shown by Atkinson who after unsuccessful attempts to reinoculate the host and produce the disease concluded the condition was a physiological disorder with the bacteria acting as secondary parasites (Atkinson, 1892). This conclusion was questioned (Earle, 1899) and proven incorrect (Smith, 1901) when inoculations of leaves and bolls resulted in typical lesions. The causal organism is a short, rod-shaped, non-spore forming, actively motile bacterium capable of producing quantities of slime (Smith, 1901). Invasion of petioles and stems results in the "blackarm" stage of the disease and invasion of the bolls results in the "boll rot" stage (Edgerton, 1912a). Infected bolls are subject to invasion by numerous filamentous fungi (Edgerton, 1912b; Ray, 1942).

The bacteria adhere to the lint (Edgerton, 1912a) and enter the seed through the basal cap (Tennyson, 1936). They have been found in the resin canals and the cotyledonary tissue (Archibald, 1927). Infected plants are the source of inoculum in the field and the bacteria are spread to surrounding plants chiefly by wind blown rain (Faulwetter, 1917, 1919) but can be spread by irrigation water (King and Parker, 1949; Shields, 1957). Insects have little if any effect in dissemination of the angular leaf spot organism (Faulwetter, 1917). When the bacteria reach the host they enter uninjured epidermis of leaves and bolls and produce lesions in 9 to

16 days (Edgerton, 1912b). Natural resistance is the only known control (Bird, 1954) but the disease can be reduced by acid delinting the seeds (Massey, 1927; Ludwig, 1922; Bain, 1939).

No reports are known of the use of antibiotics on cotton as a foliage protectant. The use of antibiotics on other plants has shown the absorption into the leaf (Daines, 1956; Anderson and Nienow, 1947) and the diffusion of an antibiotic was demonstrated on pear and apple tissue (Dunegan and Wilson, 1956). The systemic action of streptomycin is probably more important than surface protection in the field (Williams and Lockwood, 1956).

Antibiotics, while offering protection against a bacterial disease, may also produce injury to plants. Streptomycin "inserted" in young Elberta peach tree trunks caused chlorotic leaves (Dunegan and Wilson, 1956). Garden vegetables, celery and radish, were susceptible to injury by streptomycin but recovered rapidly (Marlett, 1956). Gall formation was not suppressed when antibiotics were applied to highbush blueberries but severe defoliation and reduction of yield resulted (Zuckerman, 1957).

The use of fungicides as a seed protectant is recommended for cotton (Presley, 1954; Shields, 1957). Mycostatin, when used as a postharvest protectant in peach decay tests, gave no data from which conclusions could be drawn but indications were that some reduction had been attained (Dimarco and Davis, 1957a). In a postharvest treatment of strawberries with Mycostatin, the treated berries had only 4 percent moldy fruit while the checks, hydrocooled and dry, had 31 and 37 percent, respectively (Dimarco and Davis, 1957b).

METHODS AND MATERIALS

Field Studies

Experiment I

Field studies conducted during the summers of 1954 through 1957 were performed in Northeast Texas. These studies were conducted in order to determine the effectiveness of antibiotic sprays as a means of control of angular leaf spot of cotton, Xanthomonas malvacearum.

The land for the field plots in 1954 was furnished by East Texas State College on the college farm. The soil was a sandy loam which had been used for several years in the grass adaptations plots. This land had not been fertilized. The plot was bordered by pasture on three sides with a small grain field on the fourth side.

Since no farm machines were available, the bedding was done by hand with a rake and a hoe. The beds were in rows with thirty-eight inch centers.

The cotton varieties selected were Deltapine,[1] because of its tolerance to the angular leaf spot disease, and Rowden,[2] because of its complete susceptibility to angular leaf spot. The fuzzy seeds were planted by hand in a furrow approximately two inches deep. The varieties were planted in alternating rows, the seeds were covered, and the soil was packed along the top of the bed, a common practice in this area.

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[1] Deltapine seeds furnished by Mr. Dow Porter, Superintendent of the Cotton Experiment Station, Greenville, Texas.

[2] Rowden seeds furnished by the Hurley Rowden Custom Ginning Co., Cooper, Texas.

The bacterial inoculum, a composite of races 1 and 2,[1] was maintained on potato-carrot dextrose agar (PCDA).[2] The organisms grown on PCDA, incubated at 30°C. for 48 hours, were streaked on sterile petri plates of the same medium and incubated as above. A suspension of the bacteria was made by flooding the cultures with sterile distilled water. A sterile inoculation needle was used to remove the growth from the surface of the agar and place it in suspension. The growth from one plate of each race was suspended in one-half gallon of sterile distilled water, immediately before inoculation to reduce the possible rupture of the bacterial cells due to osmosis.

Solutions of each of the five antibiotics, Penicillin Sulphate, Streptomycin, Aureomycin, Terramycin, and Neomycin[3] were prepared by weighing the sale on a microbalance in 250 mg. quantities and adding to 500 ml. of sterile distilled water to produce a 500 parts per million (ppm.) dilution. The rates of 5 ppm. and 10 ppm. were obtained by dilution of the concentrated solution.

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[1]Pure cultures of X. malvacearum, races 1 and 2 and formula for PCDA furnished through the courtesy of Dr. L. S. Bird, Pathologist, Texas A & M College, College Station, Texas.

[2]PCDA. Diced potatoes 200 gm., diced carrots 50 gm., dextrose 20 gm., magnesium sulphate 0.3 gm., calcium carbonate 0.2 gm., agar 25 gm. Add strained broth extracts of potatoes and carrots to magnesium and calcium salts, and dextrose dissolved in water. Add mixture to dissolved agar and make up to one liter.

[3]Chemicals furnished by the Research Laboratories of Merck and Co., Inc., Rahway, N. J.

Experiment II

Field study in 1955 was moved from the college land to the farm of Mr. Fred Cox, who furnished free of charge an acre of prairie black land, a clay of the Houston type. This land had been planted to cotton for several years. Mechanical equipment and operating advice were available for preparation of the soil.

The land was bedded and the seeds planted on May 1 with a two row tractor powered planter. The planting differed from the previous year in that two rows of Deltapine were alternated with two rows of Rowden. April rains provided good soil moisture and the seeds germinated and the plants grew rapidly. Four one-hundred-plant replications were treated with each antibiotic and rate. An uninoculated, untreated check and a distilled water treated and inoculated check were included in each replication. A table of random numbers (Snedecor, 1946) was used in assigning treatment and rate to each replication.

Inoculum and chemical sprays were prepared as given before. When the cotton plants were in the three leaf stage, the chemical treatments as protective sprays were applied. The sprays were applied with a three gallon Root-Lowell hand pumped, tank pressure sprayer with a nozzle adjusted to produce a 60° cone-shaped spray. A pressure of approximately 60 pounds per square inch (psi.) was maintained. The spray was directed up one side of the plant, across the top, and then up the opposite side. This was done in an attempt to simulate the action of a boom type sprayer with three nozzles per row. Each treatment was applied to all four replications, before the sprayer was rinsed thoroughly and filled with the next treatment.

The inoculum was applied from a Milwaukee carbon dioxide cartridge powered, one quart sprayer. The inoculum was applied while the plant was still wet with the protectant spray using a sweeping motion from the bottom of the plant upward. The inoculum was directed on each plant for approximately two seconds, measured by counting one thousand and one, one thousand and two. Untreated checks were sprayed with sterile distilled water and inoculated in the same manner as given above. Untreated, uninoculated plants were allowed to grow under natural environment. Observations to determine the number and intensity of diseased plants were made three weeks after inoculation since 9 to 16 days are required for lesions to develop.

Experiment III

In 1956, the study was on the same farm as in 1955. The preparation of the soil and seeding was the same as the previous year.

Experiment IV

In 1957, for the fourth time, the field study was repeated on the same farm used in 1955 and 1956. The soil was prepared and the antibiotics and rates were applied in the same manner as the two previous years. Due to the breaking of the drought in this area, good soil moisture prevailed throughout the summer.

Certified seed of the two varieties were purchased from commercial dealers. These seeds were planted in the same manner as the previous year and randomization of treatments was done as in Experiment II.

The preparation of inoculum and spray materials did not differ from

the previous experiment, but the time of application differed due to the rain which occurred at the time the plants were in the three leaf stage at which stage the chemicals were to be applied. The soil is extremely gummy when wet and by the time the ground had dried enough to permit walking on it, the plants had grown to the stage in which young bolls were being developed. Before spraying, all plants exhibiting bacterial lesions were removed. Observations were made for the number of plants showing bacterial lesions.

Xanthomonas malvacearum being a seed borne bacterium, seed treatments with antibiotics seemed to be a possible means of reducing or destroying the bacteria, thereby reducing the disease in a stand of cotton. Preliminary tests were performed (1) to determine the amount of natural infection in the seeds to be used in later experiments and the amount of infection obtained by soak inoculation, (2) to determine if the quantity of antibiotic absorbed is sufficient to inhibit bacterial growth, and (3) to determine inhibitory activity of antibiotic after storage of treated seed for one week.

Several hundred seeds were selected by reaching into a burlap bag containing approximately one-half bushel of seeds. Several hands full of seeds were removed, without regard to position in bag, for acid delinting. These seeds were immersed in concentrated sulphuric acid and stirred with a glass rod until all lint was removed from seed coats. This required approximately ten minutes. The delinted seeds were removed from the acid and placed in running tapwater and thoroughly washed for five minutes, spread on paper towels and dried.

To compare the percentage of natural infection with the percentage of infection obtained by soak inoculation, one hundred and eight seeds were surface sterilized in absolute alcohol (Archibald, 1927) for three minutes. The seed coats were removed aseptically with a sterile knife, the embryos were transferred to sterile PCDA plates with sterile forceps. Twelve plates with nine embryos per plate were incubated at 30°C. for 48 hours. One hundred and eight of the seeds were immersed in a bacterial suspension of races 1 and 2 which was prepared in the same manner as given before, and soaked for four hours. The inoculum was decanted and seeds were surface sterilized in absolute alcohol for three minutes. Upon removal from alcohol, the seed coats were aseptically removed as before, and the embryos embedded in sterile PCDA. Twelve plates with nine embryos per plate were incubated for 48 hours.

Observations of the plates revealed that only one embryo of the uninoculated seeds developed the orange-yellow colony of X. malvacearum for a 0.93 percent infection while 80 embryos of the soak inoculated seeds developed the bacterial colonies (Figure 2) denoting 73.4 percent infection.

To determine if the antibiotic was absorbed by the seeds and if the quantity was sufficient to inhibit bacterial growth, 108 seeds were immersed in solutions of Streptomycin at each of the rates of 250 ppm., 500 ppm., and 1000 ppm. and soaked for four hours. The antibiotic solutions were decanted and the seed coats were removed as before. The embryos were embedded in 12 PCDA plates with nine embryos per plate. The plates had previously been surface seeded by pouring a suspension

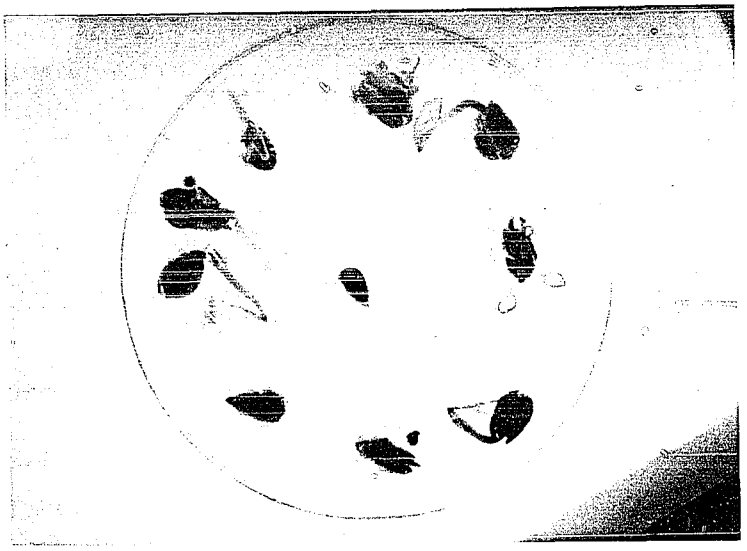


Figure 2. Bacterial growth from cotyledonary tissue from cotton seed soak inoculated with X. malvacearum, surface sterilized, excised and embedded in sterile PCDA (Photograph by Dr. Otho Spencer)

of the X. malvacearum over agar, and incubating for 48 hours.

Observations showed that the 250 ppm. of Streptomycin did not exhibit any clear zone of inhibition around the embryos. Seeds treated with 500 ppm. exhibited cleared zones of inhibition (Figure 3) from very little inhibition adjacent to two seed embryos to those embryos in which the inhibition zones had coalesced to produce a large inhibition zone. The 1000 ppm. treated seeds indicated inhibition by all embryos although there were variations in extent of area in the plates. These data indicate Streptomycin at the highest level appeared to be absorbed at a level sufficient to inhibit growth of the organisms on the surface of the embryos (Figure 4).

One hundred and eight seeds from each of the above treatments were stored at refrigerator temperature for one week. The embryos were excised as before and embedded in PCDA which was surface seeded with X. malvacearum and incubated for 48 hours.

Observations revealed that no clear zones of inhibition were present adjacent to any of the embryos, indicating the antibiotic had been altered in some way.

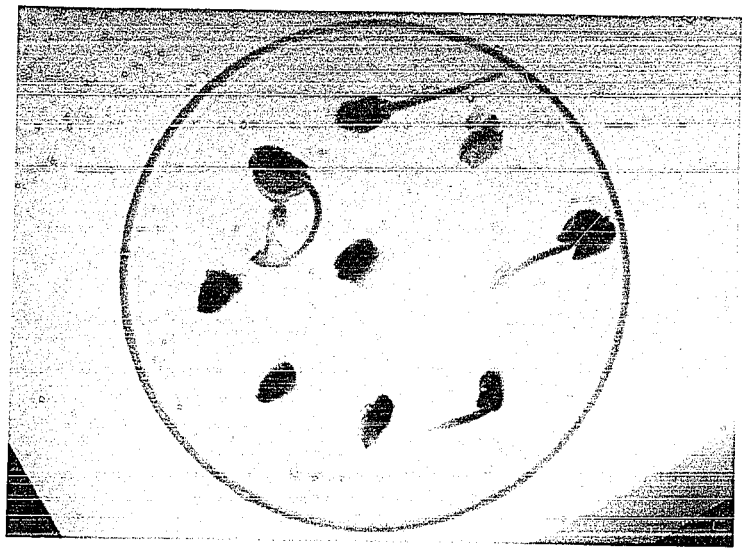
Greenhouse Studies

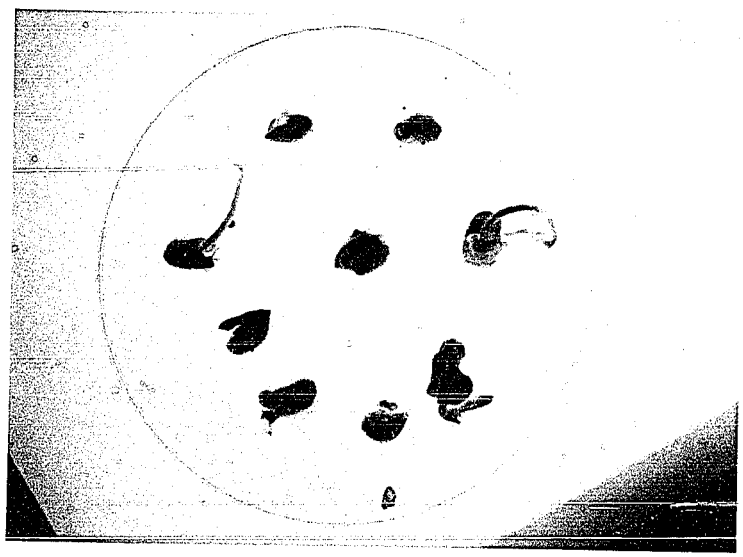
Experiment V

A preliminary experiment to develop techniques of inoculation and methods of evaluation was performed. Four thousand eight hundred seeds of each of the varieties, Deltapine and Rowden, were acid delinted, thoroughly washed and dried. The seeds of each variety were divided into two equal

Figure 3. Cleared zones of inhibition around embryo excised from cotton seed treated with 500 ppm. Streptomycin and embedded in PCDA surface seeded with X. malvacearum (Photograph by Dr. Otho Spencer)

Figure 4. Cleared zones of inhibition around embryo excised from cotton seed treated with 1000 ppm. Streptomycin and embedded in PCDA surface seeded with X. malvacearum (Photograph by Dr. Otho Spencer)





lots for further treatment. One lot of 2400 seeds of each variety was used for soak inoculation and the second lot for vacuum inoculation.

The inoculum was prepared from pure cultures of races 1 and 2 of X. malvacearum grown on PCDA. A suspension was made by flooding one agar plate of race 1 with sterile distilled water, agitating the liquid and scraping the bacteria from the surface of the agar with a sterile inoculating needle. This suspension was added to one-half gallon of sterile distilled water. A culture of race 2 was treated in the same manner and the suspension diluted to one-half gallon. The two suspensions were combined into one gallon of inoculum material.

Twenty-four hundred seeds were soak inoculated in half of the composite suspension for four hours. The inoculum was decanted and the seeds were spread to dry. They were then divided into 24 lots of 100 seeds each and packaged.

Seeds from ten packages were placed in separate containers and soaked for four hours in solutions of 5 ppm. and 10 ppm. of five antibiotics: Aureomycin, Neomycin, Penicillin, Streptomycin, and Terramycin. Seed from ten other packages were treated with the same antibiotics at the same rates in a vacuum jar to which was applied a negative pressure of 10 mm. of mercury until air bubbles ceased to appear. Seeds in the four remaining packages were soaked in sterile distilled water for four hours as checks, dried and placed in labeled packages for planting.

The second 2400 seeds were inoculated with the remaining half of the composite suspension of X. malvacearum in a vacuum jar under a negative pressure of 10 mm. of mercury until no air bubbles were observed in the suspension. This required approximately ten minutes. The seeds were

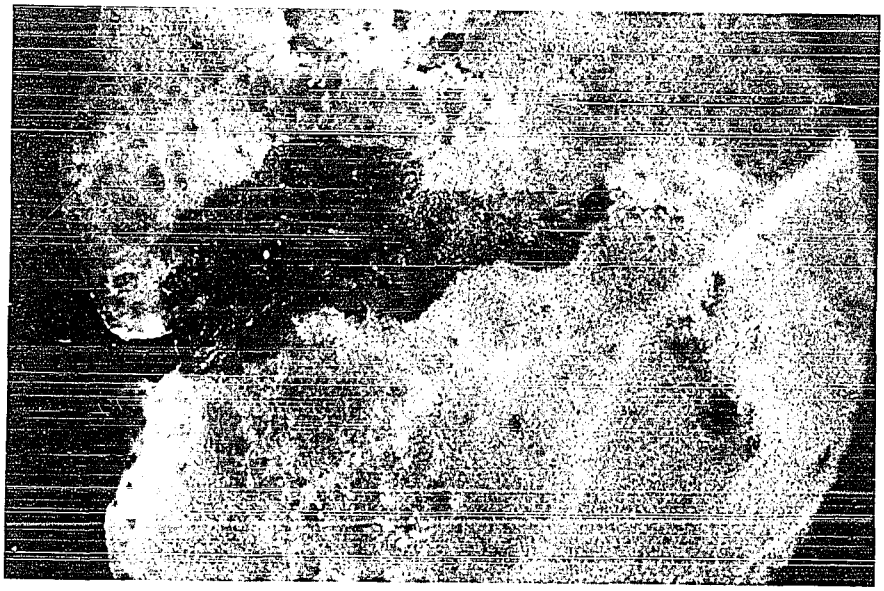
removed, dried and packaged in 24 lots of 100 seeds for treatment with antibiotics and sterile water as described above.

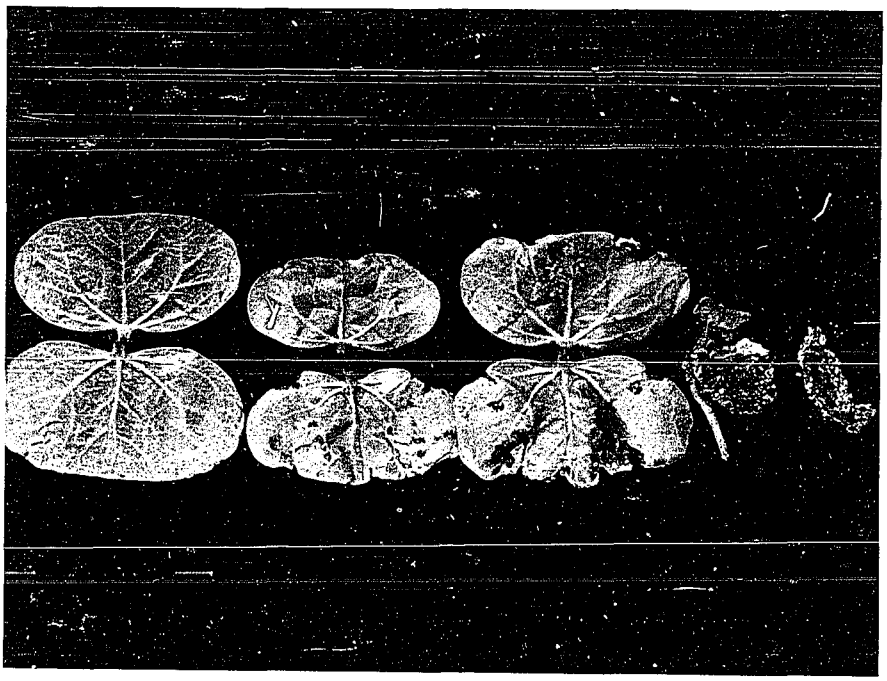
The seeds were planted in pasteurized sand in the germination room of the Iowa State College Seed Laboratory, in which a constant temperature of 80°F. ± 1° was maintained. The sand was 3 to 4 inches deep in benches four feet wide and was at a temperature of 77°F. when planted. After tap-water at 65°F. was added, the temperature of the sand was probably lower, however. Furrows for planting the seeds were made with a planting board. By pressing the board into the moist sand, furrows two inches deep and two inches apart were made. One hundred seeds were placed in each furrow, and pot labels bearing all the data as to variety, antibiotic, and rate were placed at the end of each row. The seeds were watered by the laboratory personnel who tried to maintain uniform moisture in all parts of the bench.

Acid delinted seeds usually germinate within three to four days at temperatures of 85 to 90°F. present in Texas at planting time. The seeds planted in the sand benches required 14 days to germinate because of the low temperature which prevailed. The cotyledons showed typical water-soaked lesions of angular leaf spot (Figure 5). On some the disease had developed to the extent that large areas were completely decomposed. To establish a basis for estimating the area diseased, the cotyledons were placed on a colony counting apparatus which had a light for a background and a face marked in uniform squares. Use of the counter made possible an accurate measure of diseased and healthy leaf tissue. Percentage of diseased area was first estimated without use of the counter then checked by measuring the same area with the counter. The estimate of disease could

Figure 5. Water-soaked lesion on cotyledon of cotton plant grown from soak inoculated seed

Figure 6. Disease class rating for infected cotyledons. Reading left to right: Class 1, 0 disease; Class 2, 1-10 percent diseased; Class 3, 10-40 percent diseased; Class 4, 40-90 percent diseased; Class 5, 90-100 percent diseased





be made within the ranges set for the experiment. The cotyledons were assigned to five classes (Figure 6): Class 1, zero disease (healthy); Class 2, 0 to 10 percent of cotyledon tissue diseased; Class 3, 11 to 40 percent diseased; Class 4, 41 to 90 percent diseased; and Class 5, 91 to 100 percent diseased.

Experiment VI

A more refined cotton seed treatment experiment with two organic fungicides and two antibiotics was planned using a randomized complete block design suggested by Dr. T. A. Bancroft, head of the Department of Statistics at Iowa State College. Higher germination temperatures and higher rates of treatment with chemicals more likely to provide beneficial effects were planned.

Fungicides selected were Ceresan M, a 7.7 N-(ethylmercuri)-p-toluenesulfonanilide, which is recommended as a cotton seed treatment for protection against soil fungi, and Omadine, a sodium salt of 2 pyridine-thione 1-oxide, which has demonstrated a better control of blossom blight of pears than bordeaux 2-6-100 (Hamilton and Szkolnik, 1957). Rates were 2, 4, 8 and 10 oz. and 1, 2, 4 and 8 oz. per 100 lbs. of seed, respectively (Table 1).

Antibiotics selected were Streptomycin, which has controlled other bacterial diseases (Heggested and Clayton, 1954), and Mycostatin, which has shown indications of reduction of peach decay and mold in strawberries (Dimarco and Davis, 1957a, 1957b). Both were used at 100, 250, 500 and 1000 ppm. Streptomycin was dissolved in sterile distilled water; Mycostatin in methyl alcohol (Table 1).

Table 1. Treatments[a] used on seeds of cotton varieties Deltapine and Rowden to control X. malvacearum (Experiment VI)

Treatment	Chemical form	Physical form	Rates
Ceresan M	Organic mercury fungicide	Dust	2 oz./100 lbs. seed 4 oz./100 lbs. seed 8 oz./100 lbs. seed 10 oz./100 lbs. seed
Mycostatin	Antibiotic bactericide fungicide	Powder dissolved in methyl alcohol	100 ppm. 250 ppm. 500 ppm. 1000 ppm.
Omadine	Synthetic antibiotic bactericide	Dust	1 oz./100 lbs. seed 2 oz./100 lbs. seed 4 oz./100 lbs. seed 8 oz./100 lbs. seed
Streptomycin	Antibiotic bactericide	Water soluble powder	100 ppm. 250 ppm. 500 ppm. 1000 ppm.

[a]Inoculated untreated check rows were included for each rate of each chemical and of each variety. Eight per replication.

To maintain the temperature at 85°F., a level most favorable for cotton seed germination, a greenhouse bench, with under bench heating and an attached skirt of heavy green nylon cloth enclosing the space between the bench and the floor (Figure 7), was used. The steam flow was regulated by a valve at one end of the bench. To obtain even distribution of the heat, a ten-inch electric fan was placed at one end to circulate the heated air.

Soil, prepared by mixing three parts of black loam with two parts peat and one part sand, was placed in a soil mixing machine to obtain

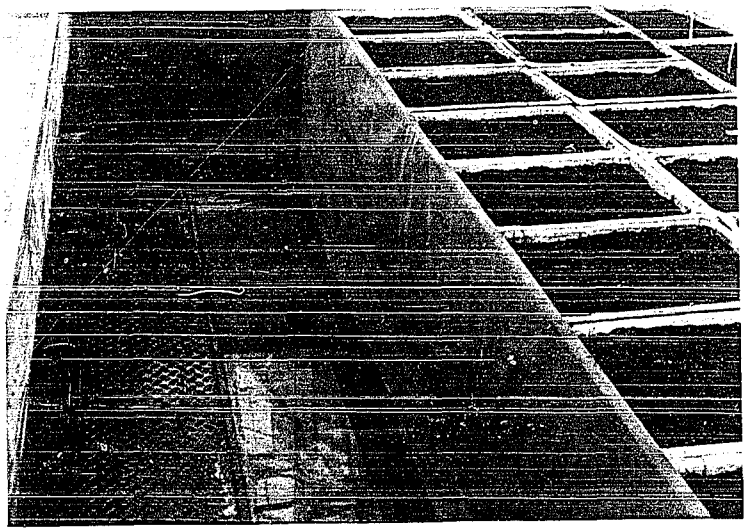


Figure 7. Skirt enclosing space under bench to hold heat to obtain high soil temperature in flats on bench

thorough mixing. After steam heating in an autoclave (Figure 8) at 15 pounds pressure for three hours, the soil was placed in galvanized flats (Figure 9) measuring 21 inches long, 13.5 inches wide and 3.75 inches deep. The flats were filled to a depth of 3.5 inches with the heated soil and placed as close as possible to each other so the heat loss would be at a minimum. Thermometers were inserted to a depth of two inches and spaced in such a manner that no more than one flat separated any two thermometers (Figure 10). The soil was watered twice daily during the heat regulating period. By reading the thermometers at regular intervals and adjusting the steam flow, a constant temperature of 85°F. was maintained. Throughout the experiment the temperature did not vary more than one degree between the flats on the bench. The soil used during the heat regulating time was discarded and new soil was prepared as before for planting the treated seed. Thermometers were maintained in the flats during the germination period.

Thirty-two hundred seeds of each variety, Deltapine and Rowden, were acid delinted with concentrated sulphuric acid and thoroughly washed. Then the seeds were divided into four lots of 1600 seeds to be inoculated.

Inoculum was prepared by flooding plates of pure cultures of X. malvacearum with sterile distilled water. A suspension was produced by agitating the water and using a sterile inoculating needle to scrape the bacterial growth from the surface of the agar. Suspensions of bacteria from one plate each of races 1 and 2 were combined and diluted with sterile distilled water to a volume of one gallon.

The four lots of seeds to be inoculated were placed in separate containers, covered with the composite suspension of X. malvacearum and

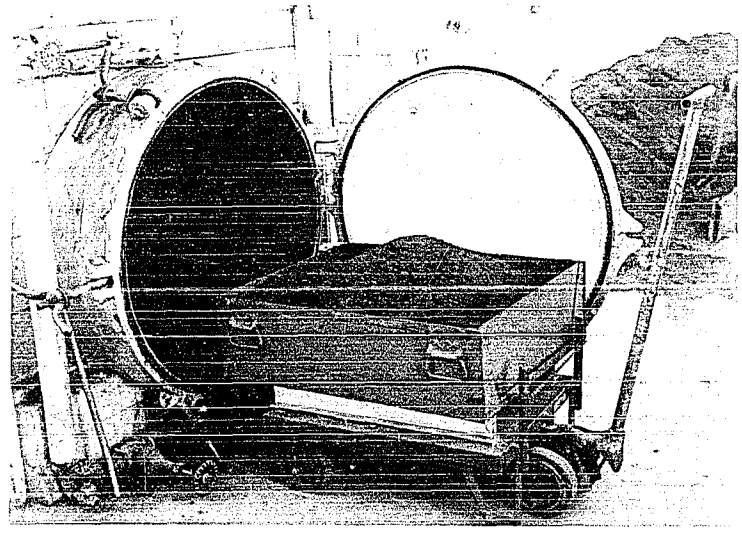


Figure 8. Soil pasteurizing apparatus with capacity for four large sterilizing flats. Soil heated at 15 pounds pressure for three hours

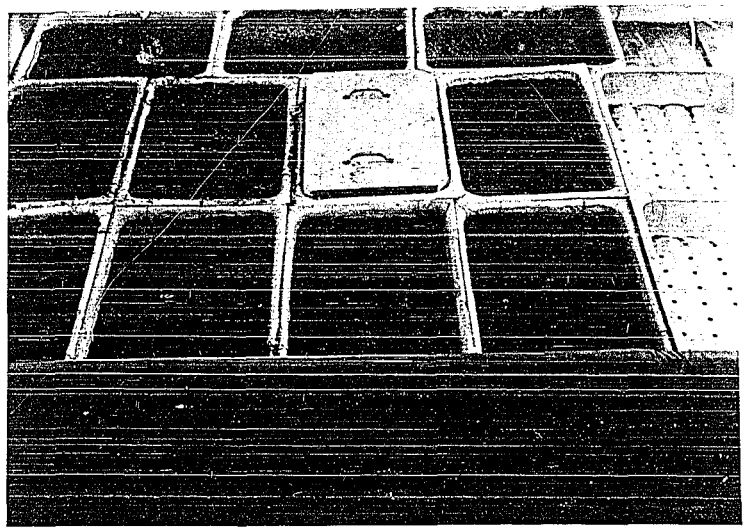
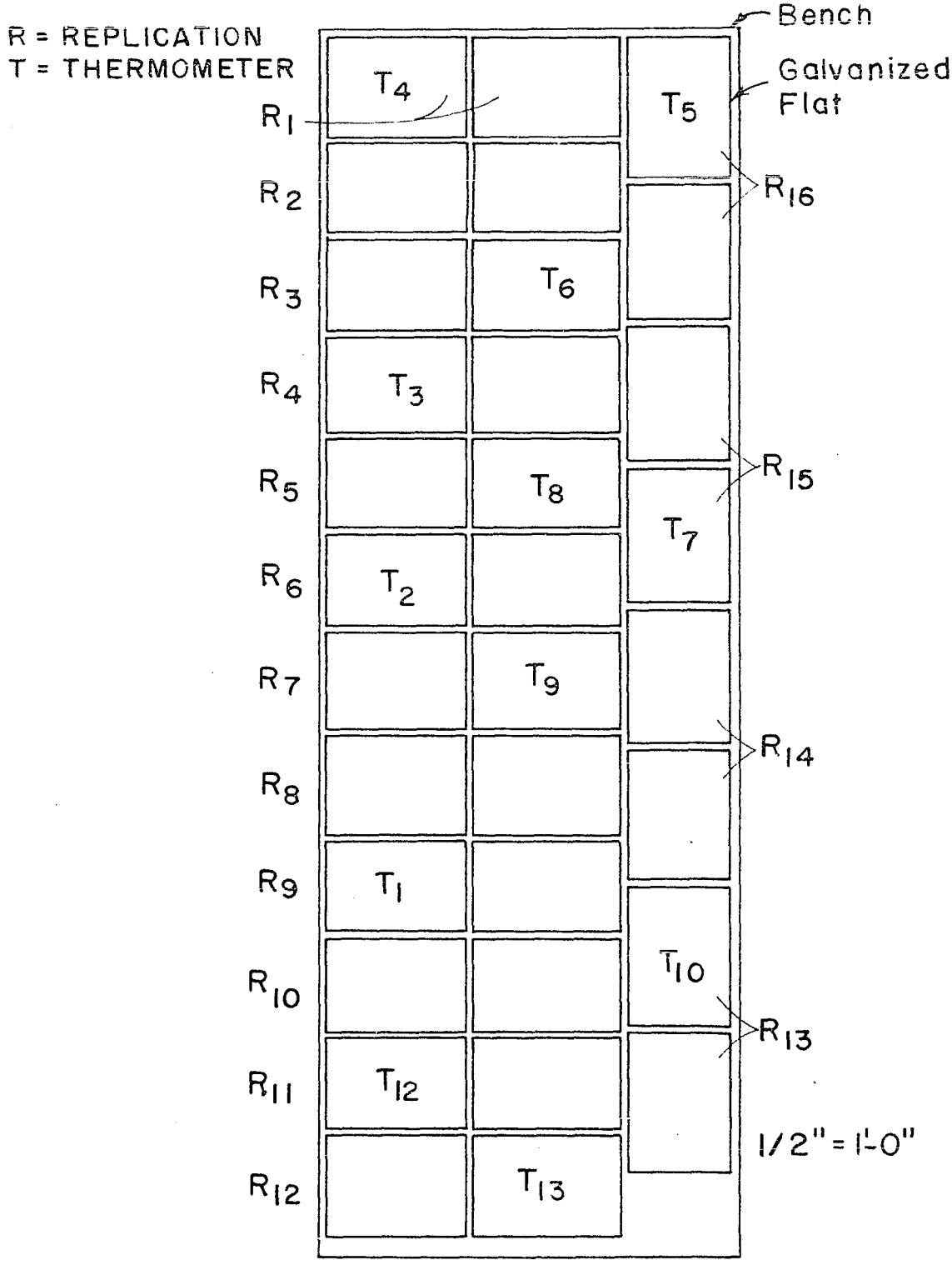


Figure 9. Arrangement of flats containing soil ready for planting with inoculated, chemically treated cotton seed

Figure 10. Replication arrangement on bench

REPLICATION ARRANGEMENT ON BENCH



allowed to soak for four hours, a period which had proved to be satisfactory in Experiment V. The seeds were removed from the inoculum, dried and divided into lots of 160 seeds each.

Seeds of each variety were treated at the various rates with the four chemicals. Weighed quantities of the powdered chemicals, Ceresan and Omadine, were placed with 160 inoculated seeds in jars on a device which caused them to be rolled continuously so the seeds became covered with the chemical. When the chemical had been distributed evenly over the seed coats and very little dust had been left in the jar, the seeds were removed and placed in packages of 10 seeds each.

The stock solution of Streptomycin was prepared by dissolving 500 mg. of the crystalline antibiotic in 500 ml. of sterile distilled water. The lower rates (100, 250 and 500 ppm.) were prepared from this 1000 ppm. solution. The previously inoculated seeds were soaked in the solutions for four hours, were removed, dried and packaged in lots of 10 seeds each.

Mycostatin, which is only slightly soluble in water, was dissolved in methanol as recommended by the manufacturer. Seeds were treated in the same manner as above.

Seeds of the remaining lots of each variety were placed in sterile distilled water and soaked for four hours as checks (zero rates of the chemicals). They were removed from the water, dried and packaged in lots of 10 seeds each.

After the soil was placed in flats, a planting board (Figure 11) was used to make 200 holes (10 x 20) one inch apart and two inches deep (Figure 12). The seeds were transferred to the holes with sterile for-

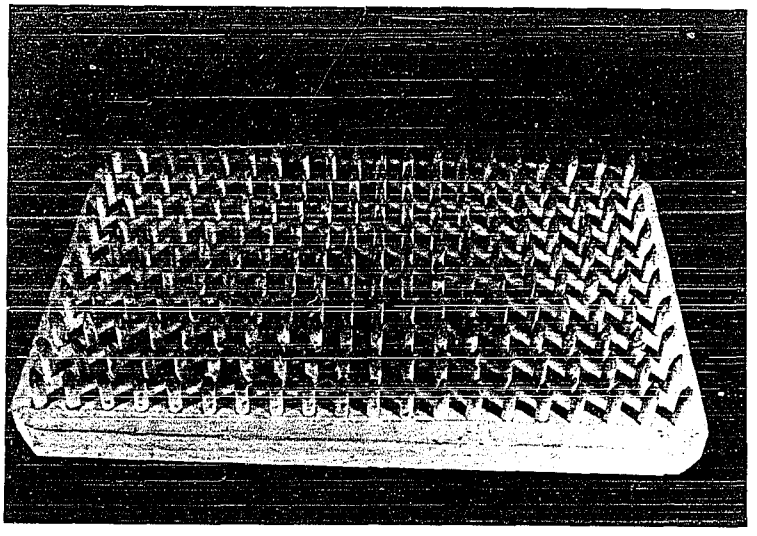


Figure 11. Planting board used for making holes in the soil for the 20 ten-hill plots

ceps, to avoid contamination and mixing of chemicals. The seeds were covered with soil and watered. The soil was watered twice daily and care was taken to provide the same amount of water to each flat.

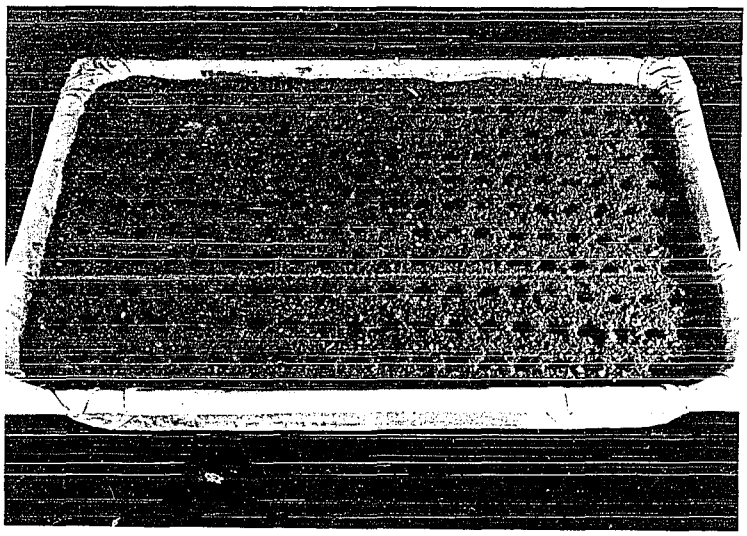


Figure 12. Flat ready for planting with cotton seeds in the 200 holes made with the planting board

RESULTS

Field Studies

Experiment I

Field studies using chemical sprays as protectants against angular leaf spot were planned for 1954 but were abandoned due to a severe grasshopper infestation at the time the cotton had reached the three leaf treatment stage. Thousands of grasshoppers were killed by poisoning but poison was not applied to the pasture bordering the plot, therefore the insects continued to invade. They ate the leaves and stalks killing all the plants in the plot. In Hunt County, 5,000 acres of cotton were destroyed during this season.

Experiment II

A second field study was conducted in 1955 in which protectant chemical foliage sprays were applied after which the plants were sprayed with a suspension of X. malvacearum. Every plant in the untreated inoculated checks had lesions on the leaves as well as the "black arm" lesions on the petioles. The same condition, however, was true of the treated plants. Therefore, the antibiotics at the rates used did not offer any protection against infection by the angular leaf spot organism.

Experiment III

The third field study was conducted during the season of 1956 with chemical sprays as protectants against X. malvacearum. Protectant sprays and inocula were not applied due to the severe drought in the area. The seeds germinated but due to the lack of moisture and the high air tem-

perature, 43 days during July and August in which the temperatures were over 100°F., the plants did not grow enough for application of the chemicals.

Experiment IV

In 1957, for the fourth time, a field test plot was prepared for the purpose of applying chemical protectant sprays to cotton plants. The climatic conditions were favorable, with sufficient rainfall to insure good soil moisture, so seeds germinated rapidly and produced good stands. Chemicals and inoculum were applied as in 1955 (Experiment II).

Observations of uninoculated, untreated plants exhibited less than 10 percent natural infection. The plants which had received a protectant spray before the inoculum of X. malvacearum was applied, showed 100 percent infection. Approximately 6 percent of the bolls showed the boll rot condition due to bacterial infection. Late August rains caused the spread of the angular leaf spot throughout the field causing severe defoliation. The chemicals at the rates used did not offer any protection to the cotton plant against the bacterium, X. malvacearum.

Greenhouse Studies

Experiment V

When all cotyledons had been classified, a disease index for each treatment was computed. The number of cotyledons in each class was multiplied by the corresponding average percentage for the class as follows: Class 1, 0 percent; Class 2, 6 percent; Class 3, 26 percent; Class 4, 66

percent; Class 5, 96 percent. Totals for the classes were added and the sum was divided by the number of plants emerged to obtain the disease index.

Examination of the disease index data from Experiment V (Table 2) revealed few, if any, significant differences between chemical treatments and between methods of inoculation. From this preliminary study, however, several useful leads were obtained.

Severity of disease on the cotyledons was such that for future experiments conditions more favorable to the cotton plants must be obtained. Too few plants emerged and too many that emerged had cotyledons so badly diseased readings were meaningless. Soil temperatures more nearly approximating those prevailing in cotton growing regions (85-90°F.) probably would reduce the time required for emergence and the severity of disease.

The badly deteriorated cotyledons on some plants and the low percentage emergence suggested the possibility that fungi, other than the bacteria, were attacking the seedlings. Use of a standard fungicide seed treatment probably would protect the seedlings and increase the stand in future studies.

Comparison of the disease indices for the untreated check rows inoculated by soaking and under vacuum revealed no significant differences (Table 2). For future studies inoculation by soaking in bacterial suspensions should be used since the method is simple and uniform and adequate infection can be obtained.

Disease readings uniformly higher than the checks were obtained on cotyledons from seeds treated with Neomycin and Penicillin. This suggested elimination of these chemicals in future studies.

Table 2. Chemicals applied by two methods to seed of two varieties of cotton following inoculation with X. malvacearum by two methods

Method of inoculation	Method of treatment	Treatment chemical	Number of plants observed	Cotyledon infection index per plant
Soak	Soak	Aureomycin	58	36.9
Soak	Vacuum	Aureomycin	105	47.5
Vacuum	Soak	Aureomycin	54	82.8
Vacuum	Vacuum	Aureomycin	63	46.3
Soak	Soak	Neomycin	29	64.5
Soak	Vacuum	Neomycin	53	58.6
Vacuum	Soak	Neomycin	53	87.7
Vacuum	Vacuum	Neomycin	39	52.0
Soak	Soak	Penicillin	52	75.2
Soak	Vacuum	Penicillin	0	--
Vacuum	Soak	Penicillin	39	57.5
Vacuum	Vacuum	Penicillin	116	44.8
Soak	Soak	Streptomycin	51	50.8
Soak	Vacuum	Streptomycin	70	44.7
Vacuum	Soak	Streptomycin	0	--
Vacuum	Vacuum	Streptomycin	63	46.5
Soak	Soak	Terramycin	79	45.6
Soak	Vacuum	Terramycin	39	56.2
Vacuum	Soak	Terramycin	49	34.8
Vacuum	Vacuum	Terramycin	128	39.5
Uninoculated ck.	Untreated	None	140	44.8
Soak	Untreated ck.	None	285	50.6
Vacuum	Untreated ck.	None	581	34.0
		Totals	2146	52.4

The ease and accuracy with which diseased cotyledons could be classified suggested continuation of this method. For an analysis of variance of the data, however, use of the total percentages for each row instead of the disease index appeared desirable. Adjustment of means to equal-sized plots would be desirable as part of a statistical analysis.

Experiment VI

Seed of cotton varieties, Deltapine and Rowden, were soak inoculated in a suspension of races 1 and 2 of X. malvacearum, were treated with two fungicides and two antibiotics at four rates and were planted in a randomized block design in a greenhouse. When the seedlings had emerged they were counted and the cotyledons were assigned to five classes on the basis of the amount of infection present. From the tabulated data (Appendix) stand totals for the different treatment combinations were assembled (Table 3), infection percentages were compiled and analyses of variance and covariance were computed by the Statistical Laboratory of Iowa State College.

An analysis of variance computed from the stand data in Table 3 revealed highly significant mean squares for varieties, and for chemicals and a significant mean square for the variety x chemical interaction (Table 4). Values for five missing plots were calculated and the degrees of freedom were reduced correspondingly (Snedecor, 1946). Seeds treated with Mycostatin failed to germinate, so this treatment was omitted from all analyses. Since the mean squares for varieties and for the variety x chemical interaction were significant, the means were compared by chemicals within varieties to discover the nature of the differences (Table 5).

Table 3. Experiment VI. Stands of cotton plants of two varieties resulting from inoculation with X. malvacearum and treatment with four chemicals at four rates, sixteen replications

Treatment	Code	Deltapine (plants)	Rowden (plants)	Total (plants)
Check	0	301	342	643
Mycostatin[a]	2			
Ceresan	1	452	459	911
Streptomycin	4	363	446	809
Omadine	3	452	471	923
Total: without check		1267	1376	2643
with check		1568	1718	3286

Code	Treatment	Variety	Rate 1	2	3	4	Total
1	Ceresan	Deltapine	110	114	105	123	452
	(2,4,8,10 oz. per	Rowden	118	114	117	110	459
	100 lbs. of seed)	Total	228	228	222	233	911
4	Streptomycin	Deltapine	94	91	87	91	363
	(100,250,500,	Rowden	114	107	113	112	446
	1000 ppm.)	Total	208	198	200	203	809
3	Omadine	Deltapine	117	115	107	113	452
	(1,2,4,8 oz. per	Rowden	102	135	121	113	471
	100 lbs. of seed)	Total	219	250	228	226	923

[a]Because of methyl alcohol injury to the seeds treated with Mycostatin, these data are omitted.

Table 4. Analysis of variance of stand data in Table 3 (Experiment VI)

	df	ss_x	ms_x	F
Total	479-5[a] = 474	1732.5917		
Replication	15	310.4583	20.6972	7.3187**
Variety	1	46.8750	46.8750	16.5735**
Chemicals[b]	3	63.9377	21.3126	7.5394**
Var. x chem.	3	27.6587	9.2196	3.2601*
Rate/chem.	9	20.5076	2.2786	0.8057
Var. x rate/chem.	9	35.8151	3.9794	1.4071
Experimental error	434	1227.3393	2.8280	

[a]Five degrees of freedom removed to correct for five plots with zero stand for which calculated values were used (Snedecor, 1946).

[b]Check included as chemical 0.

*Significant at the 5% level.

**Significant at the 1% level.

Stands significantly greater than from the untreated check were obtained from treatment with Ceresan and Omadine for the variety Deltapine only. Apparently all chemical treatments failed to improve the stands for the variety Rowden over those obtained from the untreated check. By graphing the data the significant differences in stand between the Ceresan and Omadine treated plots and their respective untreated checks for the variety Deltapine become more apparent. The absence of differences between the treated and untreated plots of the variety Rowden also become evident. The highly significant differences between the stands from the Ceresan and Omadine treated plots and those from the Streptomycin plots can be seen more easily (Figure 13).

Table 5. Comparison of mean stands from seeds of two cotton varieties treated with three chemicals (Experiment VI)

Variety	Treatment	Mean stand	Check	Ceresan	Streptomycin
Deltapine	Check	6.2708			
	Ceresan	7.0625	0.7917*		
	Streptomycin	5.6719	0.5989	1.3906**	
	Omadine	7.0625	0.7917*	0.0	-1.3906**
Rowden	Check	7.1250			
	Ceresan	7.1719	0.0469		
	Streptomycin	6.9688	0.1562	0.2031	
	Omadine	7.3594	0.2344	0.1875	0.3900

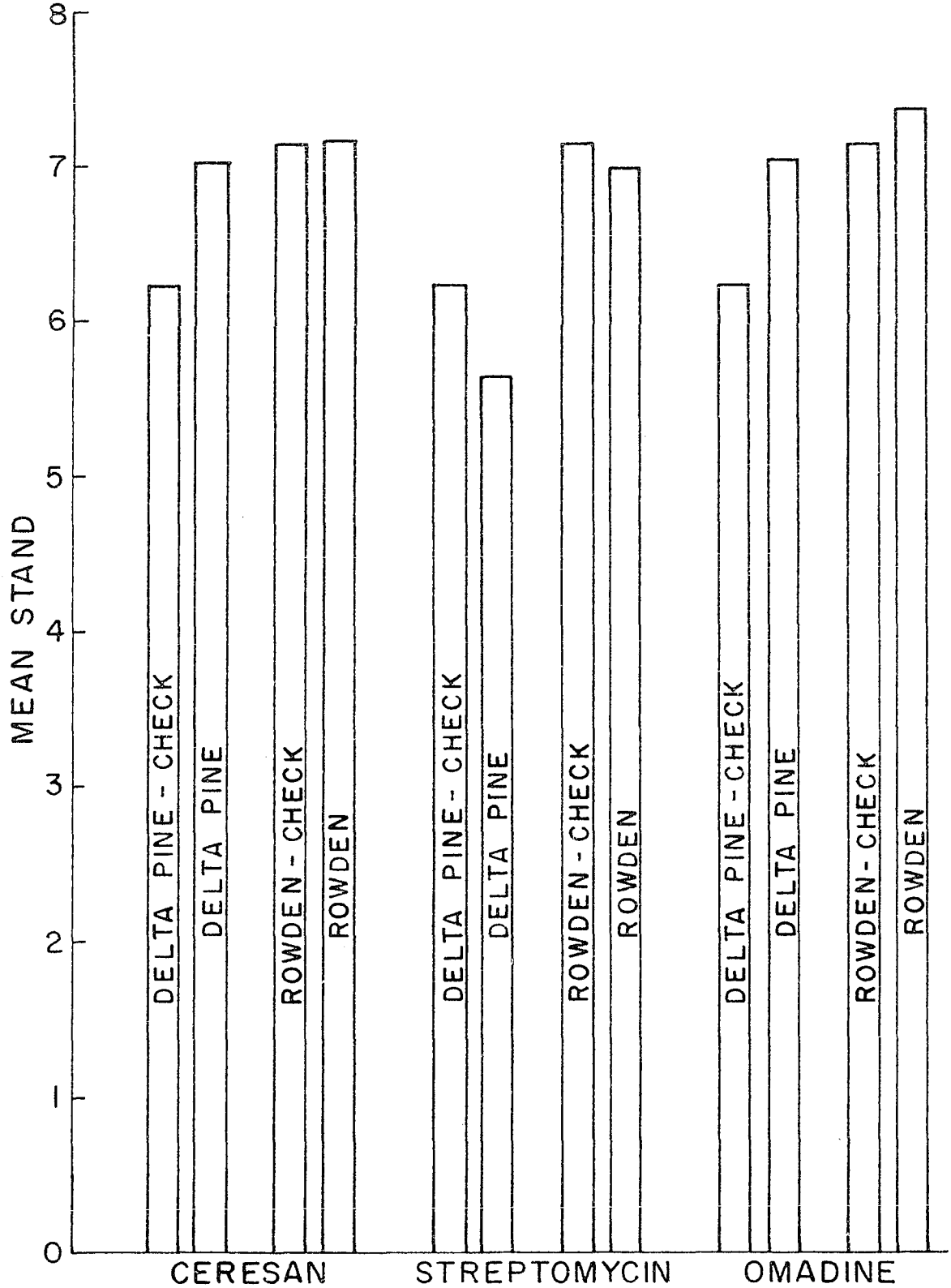
*Significant at 5% level.

**Significant at 1% level.

L.S.D. (5%) = 0.6323 for comparing check with chemical
(1%) = 0.8314
L.S.D. (5%) = 0.5856 for comparing any two chemicals
(1%) = 0.7700

Infection percentage totals (Table 6) obtained by converting infection classifications to percentages and determining the sum for each treatment combination were analyzed statistically (Table 7). The F value for variety was highly significant which indicated the varieties were responding differently to the chemical treatments. Significant mean squares were indicated for chemicals and for the variety x rate/chemical interaction. Since the percentage infection totals for the plots is dependent on the number of plants per plot, and the number of plants per plot is not the same, the method of covariance can be applied to remove the effects of the different numbers of plants per plot. Each plant had an independent and equal chance to become infected, one condition that had

Figure 13. Mean stands of cotton plants of two varieties from seed inoculated with X. malvacearum and treated with three chemicals (Experiment VI)


MEAN STAND
0
1
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DELTA PINE - CHECK
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DELTA PINE - CHECK
DELTA PINE
ROWDEN - CHECK
ROWDEN
CERESAN
STREPTOMYCIN
OMADINE

Table 6. Infection[a] of cotton cotyledons following seed inoculation with X. malvacearum and treatment with four chemicals at four rates, sixteen replications (Experiment VI)

Treatment	Deltapine	Rowden	Total
Check	8615	7978	16593
Ceresan	11386	9510	20896
Mycostatin[b]			
Streptomycin	11767	9243	21010
Omadine	13178	11246	24424
Total: without check	36331	29999	66330
with check	44946	37977	82923

Treatment	Variety	Rate 1	2	3	4	Total
Ceresan	Deltapine	2346	3188	2836	3016	11386
(2,4,8,10 oz. per	Rowden	2776	1914	2626	2194	9510
100 lbs. seed)	Total	5122	5102	5462	5210	20896
Streptomycin	Deltapine	2968	2628	2984	3187	11767
(100,250,500,	Rowden	2292	2390	2281	2280	9243
1000 ppm.)	Total	5260	5018	5265	5467	21010
Omadine	Deltapine	3580	3268	3122	3208	13178
(1,2,4,8 oz. per	Rowden	2852	3706	2880	1808	11246
100 lbs. seed)	Total	6432	6974	6002	5016	24424

[a]Figures are totals of the infection percentages for the cotyledons on all plants emerged.

[b]Because of methyl alcohol injury to the seeds treated with Mycostatin, these data are omitted.

Table 7. Analysis of variance of cotyledon infection data in Table 6 (Experiment VI)

	df	ss_y	ms_y	F
Total	479-5[a] = 474	3214344.48		
Replication	15	442565.11	29504.34	5.3069*
Variety	1	101181.17	101181.17	18.1994**
Chemicals[b]	3	62800.82	20933.61	3.7653*
Variety x chem.	3	9472.02	3157.34	0.5679
Rate/chem.	9	70061.14	7784.57	1.4002
Var. x rate/chem.	9	115405.83	12822.87	2.3064*
Experimental error	434	2412858.46	5559.58	

[a]Five degrees of freedom removed to correct for five plots with zero stand for which calculated values were used (Snedecor, 1946).

[b]Check included as chemical 0.

*Significant at the 5% level.

**Significant at the 1% level.

to exist before the data could be subjected to an analysis of covariance. Another condition that had to prevail before covariance could be used is that the number of plants per plot (x) must bear a linear relationship to the total infection (y). The regression coefficient, b = 25.4903, is a positive and significant value (Table 8). It implies that this linear relationship is consistent and the variation of y follows the variation of x in a proportional manner.

After the means had been adjusted to an equal number of plants per plot (Table 8), an analysis of variance was computed (Table 9). The presence of highly significant F values for variety, chemical, and variety x rate/chemical interaction and a significant F value for variety x chem-

Table 8. Comparison of mean infection percentages unadjusted and adjusted to equal number of plants per plot (Experiment VI)

Treatment	Mean infection percentage (unadjusted)	Mean infection percentage (adjusted)[a]	Difference
Check	172.8438	176.6138	-3.7700
Ceresan	163.2500	156.3319	6.9181
Streptomycin	164.1406	177.5358	-13.3952
Omadine	190.8125	181.7354	9.0771

[a]Regression coefficient of (y), infection percentage on (x) plant stand used to calculate adjusted mean infection percentages is: b = 25.4903

Table 9. Analysis of variance of mean infection percentages adjusted to equal number of plants per plot (Experiment VI)

	df	ss	ms	F
Variety	1	233737.71	233737.71	62.6528**
Chemical[a]	3	48452.44	16150.81	4.3292**
Var. x chem.	3	33032.05	11010.68	2.9513*
Rate/chem.	9	56518.26	6279.81	1.6833
Var. x rate/chem.	9	110233.86	12248.21	3.2831**
Error	433	1615386.44	3730.68	

[a]Check included as chemical 0.

*Significant at the 5% level.

**Significant at the 1% level.

ical interaction indicates the varieties are responding differently to the chemicals and the varieties are affected differently by the various rates of the chemicals. Comparison of means of infection percentages for the chemicals reveals Ceresan to be significantly better than the other chemicals in reducing the disease on the cotyledons and better than the untreated check. All differences for Ceresan were highly significant (Table 10). Since the varieties responded differently to the chemicals and rates

Table 10. Comparison of mean infection percentages adjusted to equal number of plants per plot (Experiment VI)

Treatment	Mean infection percentages (adjusted)	Check	Ceresan	Streptomycin
Check	176.6138			
Ceresan	156.3319	20.2819**		
Streptomycin	177.5358	-0.9220	-21.2039**	
Omadine	181.7354	-5.1216	-25.4035**	4.1996

*Significant at the 5% level.

**Significant at the 1% level.

L.S.D. (5%) = 16.3082 for check vs Ceresan, Streptomycin, Omadine
(1%) = 21.4407
L.S.D. (5%) = 15.2889 for Ceresan vs Streptomycin
L.S.D. (1%) = 20.1006 for Ceresan vs Omadine

the means were compared by rates within varieties (Tables 11, 12 and 13). On the variety Deltapine, significantly less disease was produced by treatment with Ceresan at the 2 oz. rate, and on the variety Rowden significantly less disease was produced by the 4 oz. rate (Table 11). Treatment with Streptomycin at 1000 ppm. caused significantly more disease than

Table 11. Comparison of adjusted mean infection percentages of cotton cotyledons after seed inoculation with X. malvacearum and treatment with Ceresan (Experiment VI)

Variety	Oz. per 100 lbs. seed	Mean infection percentage	Check	2 oz.	4 oz.	8 oz.
Deltapine	Check	194.1361				
	2 oz.	145.8807	48.2554*			
	4 oz.	192.1331	2.0030	-46.2524*		
	8 oz.	184.4739	9.6622	-38.5932	7.6592	
	10 oz.	167.0448	27.0913	-21.1641	25.0883	17.4291
Rowden	Check	159.0914				
	2 oz.	160.0105	-0.9191			
	4 oz.	112.5081	46.5833*	47.5024*		
	8 oz.	152.2287	6.8627	7.7818	-39.7206	
	10 oz.	136.3807	22.7107	23.6298	-23.8726	15.8481

*Significant at 5% level.

Table 12. Comparison of adjusted mean infection percentages of cotton cotyledons after seed inoculation with X. malvacearum and treatment with Streptomycin (Experiment VI)

Variety	ppm.	Mean infection percentage	Check	100 ppm.	250 ppm.	500 ppm.
Deltapine	Check	194.1361				
	100	210.2460	-16.1099			
	250	193.7754	0.3607	16.4706		
	500	222.3980	-28.2619	-12.1520	-28.6226	
	1000	228.7129	-34.5768*	-18.4669	-34.9375*	-6.3149
Rowden	Check	159.0914				
	100	136.1331	22.9583			
	250	153.4101	5.6813	-17.2770		
	500	137.0388	22.0526	-0.9057	16.3713	
	1000	138.5694	20.5220	-2.4363	14.8407	-1.5306

*Significant at 5% level.

Table 13. Comparison of adjusted mean infection percentages of cotton cotyledons after seed inoculation with X. malvacearum and treatment with Omadine (Experiment VI)

Variety	Oz. per 100 lbs. seed	Mean infection percentage	Check	1 oz.	2 oz.	4 oz.
Deltapine	Check	194.1361				
	1	211.8537	-17.7176			
	2	195.5400	-1.4039	16.3137		
	4	199.1601	-5.0240	12.6936	-3.6201	
	8	194.9763	-0.8402	16.8774	-0.5637	4.1838
Rowden	Check	159.0914				
	1	166.2492	-7.1578			
	2	191.0521	-31.9607	-24.8029		
	4	161.7311	-2.6397	4.5181	29.3210	
	8	107.4763	51.6151*	58.7729*	83.5758*	54.2548*

*Significant at 5% level. See (1) and (2) below:

(1) To compute L.S.D. (5%) for check vs chemical the following equation was used:

$$t_{0.05,\,df434} \times \sqrt{S_D^2}$$

$$S_D^2 = S_{y.x}^2 \left[\frac{1}{n_1} + \frac{1}{n_2} + \frac{(\bar{x}_1 - \bar{x}_2)^2}{SSE_x} \right]$$

$$= 3730.68 \left[\frac{1}{48} + \frac{1}{16} + \frac{(\bar{x}_1 - \bar{x}_2)^2}{1227.3393} \right]$$

where, $n_1 = 48$, the number of check plots

$n_2 = 16$, the number of chemically treated plots

(2) $$t_{0.05,\,df434} \times \sqrt{S_D^2}$$

$$S_D^2 = S_{y.x}^2 \left[\frac{2}{n} + \frac{(\bar{x}_1 - \bar{x}_2)^2}{SSE_x} \right]$$

$$= 3730.68 \left[\frac{2}{16} + \frac{(\bar{x}_1 - \bar{x}_2)^2}{1227.3393} \right]$$

the 250 ppm. rate and the check on the cotyledons of Deltapine, but the chemical was ineffective at all other rates on Deltapine and at all rates on Rowden (Table 12). Treatment with Omadine at all rates was ineffective in reducing disease on Deltapine, but at the highest rate of 8 oz. per 100 lbs. of seed significantly less disease appeared on the cotyledons of Rowden seedlings than for all other rates and the check (Table 13).

The different responses of the two varieties to the three chemicals and their rates can be seen more easily when line graphs are constructed from the data in Table 14. The only rate of all three chemicals effective in causing a significant reduction in disease for the variety Deltapine was the 2 oz. rate of Ceresan. For Rowden the 4 oz. rate of Ceresan and the 8 oz. rate of Omadine caused a significant reduction in disease on the cotyledons (Figure 14).

In Experiment VI, both the stand and the percentage infection per plot are affected by the factors variety and chemical, so it is not possible to estimate the total infection free from the effect of number of plants per plot (Snedecor, 1946). Since no growth competition occurred in the experiment, some idea can be gained as to which chemical gave the best results and which variety responded the better to the conditions of the experiment by examining the differences among the adjusted means of the varieties and the chemicals. By constructing line graphs of the data so each chemical is separated with its rates, the different responses of the varieties becomes more apparent (Figure 15). The graph for Ceresan shows readily the lower disease level resulting from treating Deltapine with the 2 oz. rate and Rowden with the 4 oz. rate. The Streptomycin graph shows the increase in disease on Deltapine resulting from treatment with 1000

Table 14. Mean infection percentages of cotton cotyledons after seed inoculation with X. malvacearum and treatment with three chemicals adjusted to equal number of plants per plot (Experiment VI)[a]

Chemical	Variety	Rate	$\bar{x}$ Mean stand	x Dev. from mean	bx Correction factor	$\bar{y}$ Mean infection percentage	$\hat{y}$ Adjusted mean infection percentage
Ceresan	Deltapine	2 oz.	6.8750	0.0292	0.7443	146.6250	145.8807
		4 oz.	7.1250	0.2792	7.1169	199.2500	192.1331
		8 oz.	6.5624	-0.2834	-7.2239	177.2500	184.4739
		10 oz.	7.6875	0.8417	21.4552	188.5000	167.0448
	Rowden	2 oz.	7.3750	0.5292	13.4895	173.5000	160.0105
		4 oz.	7.1250	0.2792	7.1169	119.6250	112.5081
		8 oz.	7.3125	0.4667	11.8963	164.1250	152.2287
		10 oz.	6.8750	0.0292	0.7443	137.1250	136.3807
Streptomycin	Deltapine	100 ppm.	5.8750	-0.9708	-24.7460	185.5000	210.2460
		250 ppm.	5.6875	-1.1583	-29.5254	164.2500	193.7754
		500 ppm.	5.4375	-1.4083	-35.8980	186.5000	222.3980
		1000 ppm.	5.6875	-1.1583	-29.5254	199.1875	228.7129

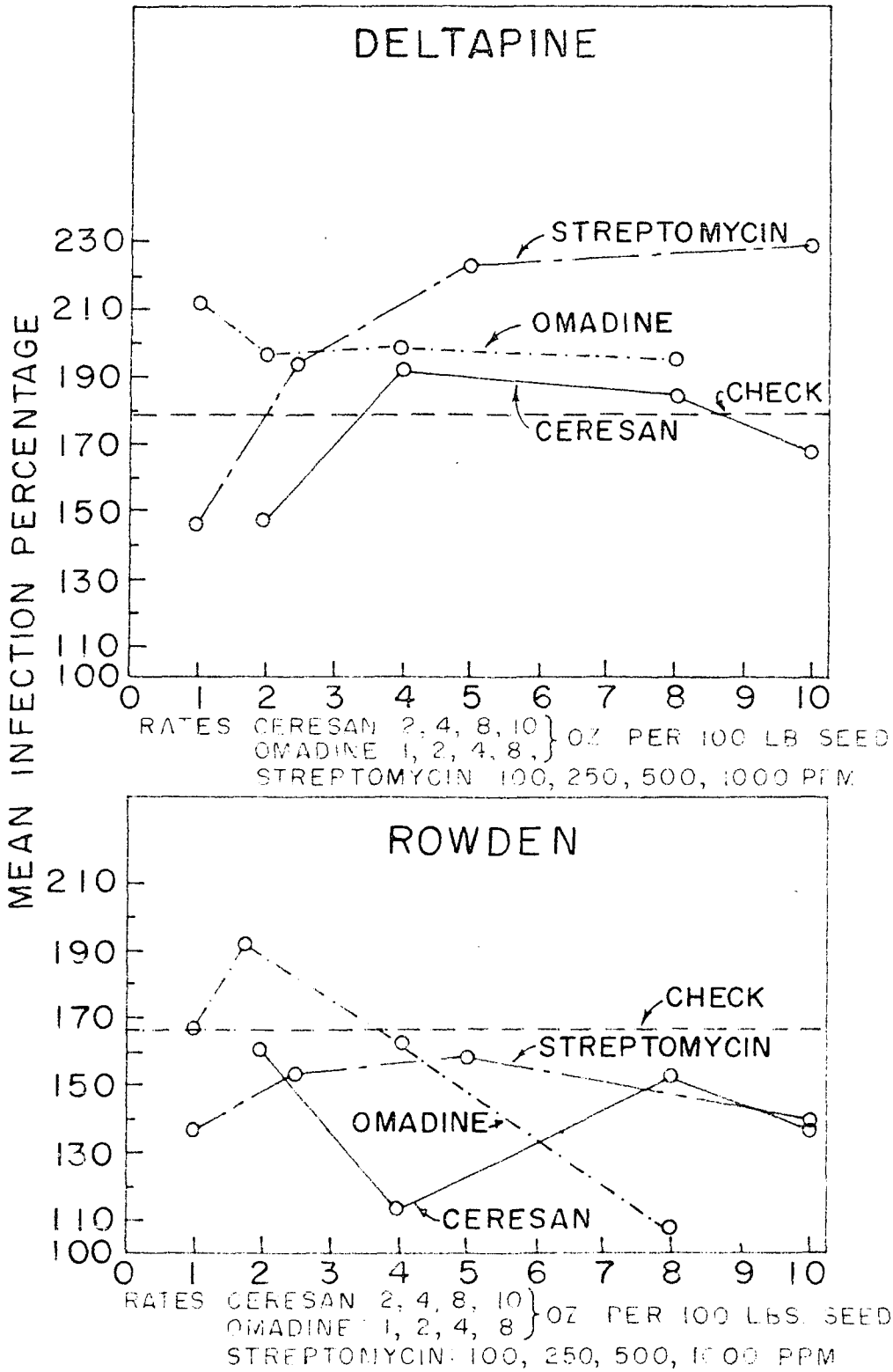
[a] $\bar{\bar{x}} = 6.8458$
Mean of stands - check (Deltapine) = 6.2708
Mean of stands - check (Rowden) = 7.1250
L.S.D. for comparing check with chemical = 0.9562 (5%)
= 1.2572 (1%)
L.S.D. for comparing any two chemicals = 1.1711 (5%)
= 1.5398 (1%)
b = 25.4903, the regression equation of (y) on (x)

$\hat{y}$ = adjusted mean = $\bar{y} - b(\bar{x} - \bar{\bar{x}})$
$= \bar{y} - 25.4903(\bar{x} - 6.8458)$

Table 14 (Continued)

Chemical	Variety	Rate	$\bar{x}$ Mean stand	x Dev. from mean	bx Correction factor	$\bar{y}$ Mean infection percentage	$\hat{y}$ Adjusted mean infection percentage
Streptomycin	Rowden	100 ppm.	7.1250	0.2792	7.1169	143.2500	136.1331
		250 ppm.	6.6875	-0.1583	-4.0351	149.3750	153.4101
		500 ppm.	7.0625	0.2167	5.5237	142.5625	137.0388
		1000 ppm.	7.0000	0.1542	3.9306	142.5000	138.5694
Omadine	Deltapine	1 oz.	7.3125	0.4667	11.8963	223.7500	211.8537
		2 oz.	7.1875	0.3417	8.7100	204.2500	195.5400
		4 oz.	6.6875	-0.1583	-4.0351	195.1250	199.1601
		8 oz.	7.0625	0.2167	5.5237	200.5000	194.9763
	Rowden	1 oz.	6.3750	-0.4708	12.0008	178.2500	166.2492
		2 oz.	8.4375	1.5917	40.5729	231.6250	191.0521
		4 oz.	7.5625	0.7167	18.2689	180.0000	161.7311
		8 oz.	7.0625	0.2167	5.5237	113.0000	107.4763

Figure 14. Comparison by variety of adjusted mean infection percentages of cotyledons of two varieties of cotton from seeds inoculated with X. malvacearum and treated with three chemicals (Experiment VI)


DELTAPINE
MEAN INFECTION PERCENTAGE
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110
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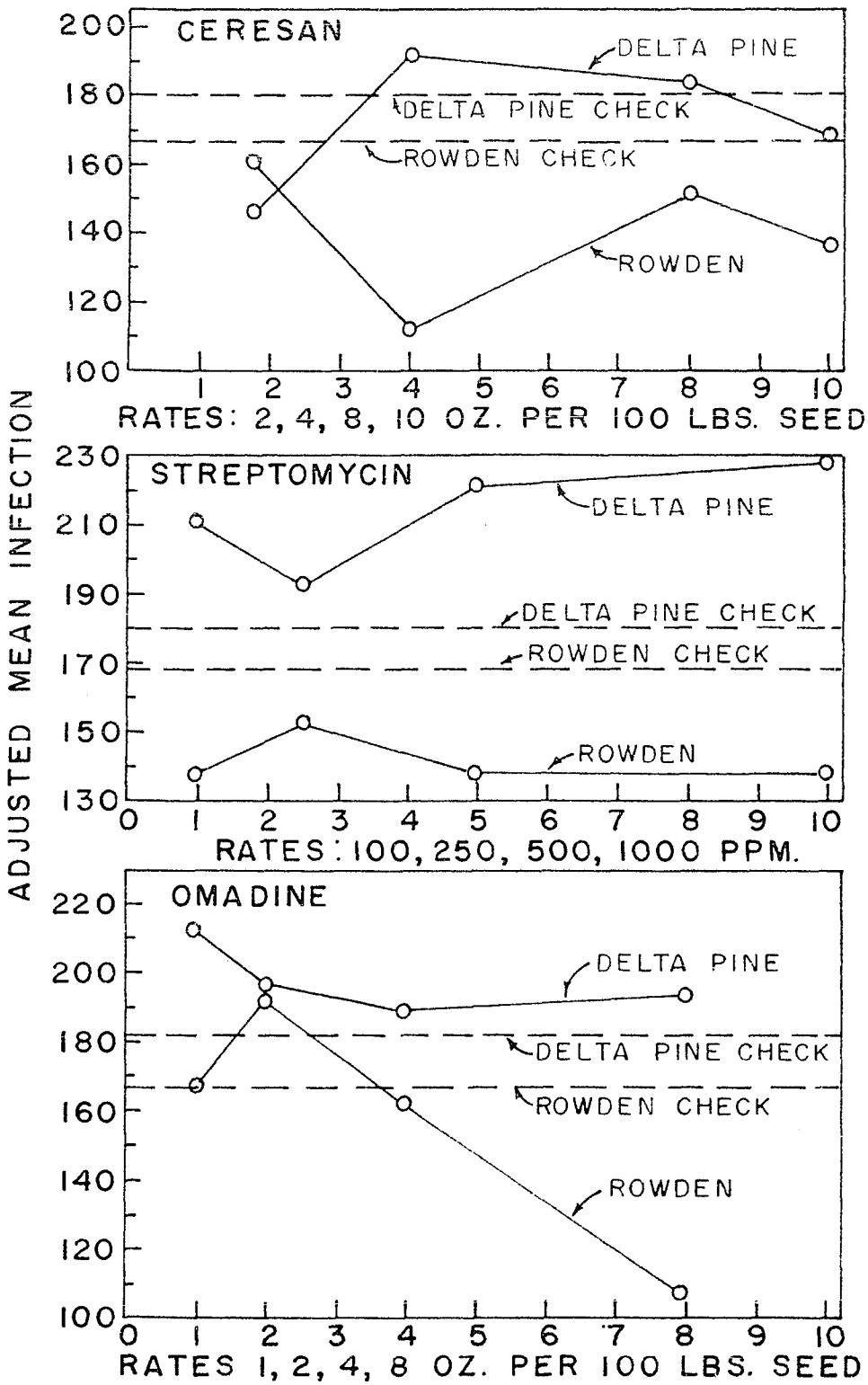
0 1 2 3 4 5 6 7 8 9 10
STREPTOMYCIN
OMADINE
CHECK
CERESAN
RATES CERESAN 2, 4, 8, 10
OMADINE 1, 2, 4, 8,
OZ PER 100 LB SEED
STREPTOMYCIN 100, 250, 500, 1000 PPM
ROWDEN
210
190
170
150
130
110
100
0 1 2 3 4 5 6 7 8 9 10
CHECK
STREPTOMYCIN
OMADINE
CERESAN
RATES CERESAN 2, 4, 8, 10
OMADINE 1, 2, 4, 8
OZ PER 100 LBS. SEED
STREPTOMYCIN 100, 250, 500, 1000 PPM

Figure 15. Comparison by chemical of adjusted mean infection percentages of cotton cotyledons after seed inoculation with X. malvacearum and treatment with three chemicals (Experiment VI)


CERESAN
DELTA PINE
DELTA PINE CHECK
ROWDEN CHECK
ROWDEN
200
180
160
140
120
100
1 2 3 4 5 6 7 8 9 10
RATES: 2, 4, 8, 10 OZ. PER 100 LBS. SEED
STREPTOMYCIN
DELTA PINE
DELTA PINE CHECK
ROWDEN CHECK
ROWDEN
230
210
190
170
150
130
0 1 2 3 4 5 6 7 8 9 10
RATES: 100, 250, 500, 1000 PPM.
OMADINE
DELTA PINE
DELTA PINE CHECK
ROWDEN CHECK
ROWDEN
220
200
180
160
140
120
100
0 1 2 3 4 5 6 7 8 9 10
RATES 1, 2, 4, 8 OZ. PER 100 LBS. SEED
ADJUSTED MEAN INFECTION

ppm. The absence of any noticeable effect of Streptomycin treatment on Rowden also becomes evident. The graph for Omadine shows the lack of any significant effects of this chemical on the variety Deltapine. On Rowden, however, the significant reduction of disease by treatment with Omadine at the 8 oz. rate is easily seen.

From these observations and within the restrictions imposed by the interrelated nature of some of the factors, it appears that the chemical Ceresan produced the most satisfactory results in that stands of Deltapine were greater on the treated plots and the percentage of infection was less. Ceresan treatment also resulted in less disease for the variety Rowden.

Of the two varieties, Deltapine showed more favorable response to seed treatment than Rowden in that treatment with 2 oz. of Ceresan per 100 lbs. of seed reduced the percentage of the cotyledon surface affected by the bacterium and increased the stand.

DISCUSSION

That the antibiotics used as foliage sprays in the field experiments failed to prevent infection of the cotton plants by the bacterium X. malvacearum should not be taken as evidence against these or any other chemical protectants. The rates of five and ten parts per million were much too low. Further attempts to use the same antibiotics should employ rates between 250 and 1000 ppm. Preliminary studies to establish those rates sufficiently high to control the disease and yet not so high as to cause phytotoxic reactions will need to be conducted. Certainly, tests in vitro cannot be regarded as dependable bases for selecting treatment rates for sprays in the field. This point of view concurs with the experience and viewpoints expressed by others. The relatively high rates of antibiotic used for controlling wildfire disease of tobacco (Heggested and Clayton, 1954) suggests the higher rates proposed above may be effective.

Many other studies should be undertaken with seed treatment chemicals to find those capable of greater stand increases and disease reductions than those used in Experiment VI. The fact that Ceresan, used presently as a seed protectant, can reduce the disease on the cotyledons opens a number of possibilities. Would a longer period between time of treatment and time of planting allow more of the organic mercury to enter the seeds and further reduce the disease? Would such a longer period result in reduced disease in Rowden? Why the difference in reaction to treatment between the two varieties? Why did Rowden require twice as much Ceresan (4 oz.) for disease reduction as was required for Deltapine? Perhaps

some morphological differences between the seeds can be found to account for the different reactions. The fact that the seeds of Rowden are about one-third larger than those of Deltapine might be worth regarding as a starting point.

Failure of any treatment to cause an increase in stand of Rowden cannot be explained entirely by assuming no chemical entered the seeds because sufficient antibiotic to cause inhibition in vitro was absorbed at the higher rates according to the tests conducted. Perhaps much higher rates can be applied safely to seeds of Rowden than to Deltapine.

Does the fact that the 10 oz. rate of Omadine was effective in reducing cotyledon infection in Rowden suggest a different physiological reaction in this variety as compared to Deltapine? Why was Omadine entirely ineffective on Deltapine so far as cotyledon infection was concerned, yet was effective at a low rate for increasing stand in this same variety? Why did Ceresan increase stand and reduce cotyledonary infection in Deltapine yet only reduced cotyledonary infection in Rowden?

Phytotoxic effects were observed on seedlings of both varieties for the two higher rates of treatment with Ceresan. Several questions arise about these observations. Would the plants have grown out of the abnormal reactions if they had been allowed to continue growth? How much higher rates could have been used safely in the case of Omadine, for example?

The variety Rowden has been considered one of the better varieties for northeastern Texas for many years. Its susceptibility to angular leaf spot has led growers and breeders to search for varieties more tolerant to this disease. Deltapine is regarded as more tolerant (Bird)[1] and is

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[1]Bird, L. S., Pathologist, Texas A & M College, College Station, Texas. Private communication. 1958.

replacing Rowden as are several other varieties. In spite of the field performance of Deltapine it was more heavily infected in the greenhouse seed treatment experiment than Rowden. Obviously, field tolerance is one thing and resistance to seed bourne bacteria another.

SUMMARY AND CONCLUSIONS

1. Rates of 5 and 10 ppm. were not high enough to control angular leaf spot of cotton with Aureomycin, Penicillin, Neomycin, Terramycin and Streptomycin as foliar sprays in field tests in Texas in 1954 and 1957.
2. Significant increases in stand and decreases in cotyledonary infection over the untreated check resulted from treatment of acid delinted seeds of Deltapine cotton with two ounces of Ceresan per 100 lbs. of seed following soak inoculation with Xanthomonas malvacearum in a replicated greenhouse experiment.
3. A significant decrease in cotyledonary infection over the untreated check resulted from treatment of acid delinted seeds of Rowden cotton with four ounces of Ceresan per 100 lbs. of seed following soak inoculation with Xanthomonas malvacearum in a replicated greenhouse experiment.
4. A significant decrease in cotyledonary infection over the untreated check resulted from treatment of acid delinted seeds of Rowden cotton with eight ounces of Omadine per 100 lbs. of seed after inoculation with Xanthomonas malvacearum in a replicated greenhouse experiment.
5. A significant increase in cotyledonary infection over the untreated check resulted from treatment of acid delinted seeds of Deltapine cotton with 1000 ppm. of Streptomycin per 100 lbs. of seed after inoculation with Xanthomonas malvacearum in a replicated greenhouse experiment.
6. Ceresan was better than Streptomycin and Omadine for increasing stand

and decreasing disease in the cotton variety Deltapine when used as a treatment for acid delinted seeds previously inoculated with Xanthomonas malvacearum in a replicated greenhouse experiment.

7. The variety Deltapine responded better than variety Rowden to Ceresan and Omadine when used as seed treatment chemicals on acid delinted seed previously inoculated with Xanthomonas malvacearum.
8. Additional field and greenhouse experiments should be conducted to determine the best chemicals and rates for cotton seed treatment to control angular leaf spot infection on cotyledons, increase field stands and reduce field infection.

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APPENDIX: RAW DATA

V_1C_1 - Deltapine - Ceresan
(2 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	204
2	8	136
3	8	176
4	5	100
5	7	184
6	7	98
7	5	140
8	2	44
9	7	132
10	7	118
11	8	196
12	8	150
13	6	112
14	10	260
15	7	132
16	7	164
Totals	110	2346

V_1C_2 - Deltapine - Ceresan
(4 oz./100 lbs. seeds)

Rep	Plants	Total
1	6	192
2	10	240
3	9	348
4	7	244
5	4	168
6	9	348
7	4	148
8	3	136
9	10	314
10	6	106
11	6	152
12	9	188
13	8	196
14	6	100
15	8	212
16	9	96
Totals	114	3188

V_1C_3 - Deltapine - Ceresan
(8 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	176
2	9	188
3	5	80
4	5	120
5	8	296
6	10	320
7	3	76
8	1	32
9	8	256
10	6	192
11	6	140
12	8	236
13	8	196
14	8	196
15	7	204
16	5	128
Totals	105	2836

V_1C_4 - Deltapine - Ceresan
(10 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	164
2	8	216
3	6	252
4	9	228
5	8	150
6	9	208
7	9	208
8	3	96
9	6	212
10	9	222
11	6	132
12	9	162
13	10	154
14	7	198
15	7	164
16	9	250
Totals	123	3016

V_1O_1 - Deltapine - Omadine
(1 oz./100 lbs. seeds)

Rep	Plants	Total
1	10	340
2	7	364
3	9	248
4	5	200
5	9	308
6	6	226
7	4	148
8	6	252
9	8	256
10	6	180
11	8	156
12	10	144
13	7	424
14	6	126
15	7	124
16	9	84
Totals	117	3580

V_1O_2 - Deltapine - Omadine
(2 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	158
2	7	244
3	9	268
4	7	118
5	8	176
6	7	344
7	7	144
8	6	172
9	8	196
10	8	330
11	6	140
12	6	220
13	8	276
14	5	140
15	8	198
16	7	144
Totals	115	3268

V_1O_3 - Deltapine - Omadine
(4 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	296
2	3	216
3	9	348
4	8	236
5	6	172
6	5	140
7	5	140
8	6	180
9	7	224
10	9	168
11	6	212
12	10	234
13	7	144
14	8	216
15	2	64
16	8	132
Totals	107	3122

V_1O_4 - Deltapine - Omadine
(8 oz./100 lbs. seeds)

Rep	Plants	Total
1	9	382
2	8	356
3	6	172
4	7	118
5	8	316
6	6	132
7	9	248
8	9	308
9	5	100
10	7	218
11	4	88
12	9	208
13	7	164
14	5	180
15	7	98
16	7	120
Totals	113	3208

V_1S_1 - Deltapine - Streptomycin (100 ppm.)

Rep	Plants	Total
1	8	396
2	7	124
3	5	180
4	5	220
5	6	172
6	6	192
7	5	160
8	4	68
9	6	152
10	5	128
11	7	164
12	5	48
13	6	312
14	4	108
15	8	278
16	7	266
Totals	94	2968

V_1S_2 - Deltapine - Streptomycin (250 ppm.)

Rep	Plants	Total
1	7	278
2	7	184
3	6	164
4	4	62
5	5	120
6	8	224
7	3	76
8	5	102
9	3	116
10	6	180
11	6	172
12	4	208
13	8	316
14	7	198
15	5	134
16	7	94
Totals	91	2628

V_1S_3 - Deltapine - Streptomycin (500 ppm.)

Rep	Plants	Total
1	8	476
2	4	128
3	8	316
4	5	380
5	10	248
6	7	326
7	2	84
8	3	64
9	1	52
10	6	166
11	6	86
12	5	100
13	6	152
14	4	128
15	5	160
16	7	118
Totals	87	2984

V_1S_4 - Deltapine - Streptomycin (1000 ppm.)

Rep	Plants	Total
1	7	178
2	8	396
3	9	308
4	5	260
5	5	200
6	5	62
7	5	300
8	6	199
9	6	232
10	6	146
11	8	150
12	4	128
13	6	232
14	1	162
15	7	198
16	3	36
Totals	91	3187

V_2C_1 - Rowden - Ceresan
(2 oz./100 lbs. seeds)

Rep	Plants	Total
1	7	138
2	9	136
3	7	158
4	7	264
5	7	224
6	8	156
7	6	100
8	3	56
9	9	452
10	9	162
11	7	242
12	8	110
13	9	268
14	8	196
15	5	50
16	9	64
Totals	118	2776

V_2C_2 - Rowden - Ceresan
(4 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	190
2	10	168
3	7	144
4	8	136
5	8	112
6	8	150
7	3	36
8	2	64
9	8	170
10	9	138
11	8	176
12	6	100
13	9	142
14	6	24
15	7	94
16	7	70
Totals	114	1914

V_2C_3 - Rowden - Ceresan
(8 oz./100 lbs. seeds)

Rep	Plants	Total
1	7	126
2	8	236
3	10	234
4	10	260
5	3	70
6	6	120
7	7	158
8	1	0
9	7	200
10	7	146
11	9	256
12	9	136
13	9	176
14	9	348
15	7	98
16	8	62
Totals	117	2626

V_2C_4 - Rowden - Ceresan
(10 oz./100 lbs. seeds)

Rep	Plants	Total
1	5	42
2	6	74
3	2	44
4	9	248
5	9	208
6	9	262
7	5	120
8	5	140
9	5	134
10	6	74
11	7	178
12	7	84
13	9	176
14	9	204
15	7	82
16	10	124
Totals	110	2194

V_2O_1 - Rowden - Omadine
(1 oz./100 lbs. seeds)

Rep	Plants	Total
1	8	164
2	6	146
3	7	204
4	4	148
5	7	144
6	7	284
7	6	252
8	3	136
9	5	160
10	10	248
11	5	100
12	6	134
13	10	328
14	9	240
15	6	140
16	3	24
Totals	102	2852

V_2O_2 - Rowden - Omadine
(2 oz./100 lbs. seeds)

Rep	Plants	Total
1	10	380
2	9	204
3	8	156
4	9	248
5	8	296
6	8	336
7	5	114
8	9	230
9	7	284
10	7	132
11	10	320
12	9	262
13	10	202
14	9	224
15	9	184
16	8	134
Totals	135	3706

V_2O_3 - Rowden - Omadine
(4 oz./100 lbs. seeds)

Rep	Plants	Total
1	9	236
2	7	164
3	9	248
4	8	176
5	8	216
6	10	164
7	3	116
8	6	92
9	8	216
10	8	236
11	9	188
12	7	244
13	9	176
14	5	48
15	7	140
16	8	220
Totals	121	2880

V_2O_4 - Rowden - Omadine
(8 oz./100 lbs. seeds)

Rep	Plants	Total
1	7	146
2	7	60
3	9	148
4	5	60
5	5	80
6	8	132
7	8	176
8	5	80
9	2	24
10	5	74
11	8	210
12	8	100
13	9	124
14	7	72
15	10	160
16	10	162
Totals	113	1808

V_2S_1 - Rowden - Streptomycin (100 ppm.)

Rep	Plants	Total
1	9	182
2	6	186
3	7	224
4	7	144
5	7	68
6	8	92
7	4	108
8	3	56
9	9	184
10	7	132
11	5	140
12	7	92
13	9	144
14	9	170
15	9	190
16	8	180
Totals	114	2292

V_2S_2 - Rowden - Streptomycin (250 ppm.)

Rep	Plants	Total
1	6	146
2	6	152
3	8	184
4	3	76
5	8	72
6	9	92
7	8	92
8	2	64
9	7	312
10	9	170
11	6	220
12	9	136
13	5	160
14	6	146
15	9	182
16	6	186
Totals	107	2390

V_2S_3 - Rowden - Streptomycin (500 ppm.)

Rep	Plants	Total
1	7	226
2	8	124
3	6	112
4	8	196
5	9	182
6	5	76
7	8	176
8	7	143
9	4	102
10	5	80
11	7	158
12	5	42
13	8	236
14	9	150
15	9	224
16	8	54
Totals	113	2281

V_2S_4 - Rowden - Streptomycin (1000 ppm.)

Rep	Plants	Total
1	6	80
2	7	120
3	6	152
4	7	204
5	7	120
6	6	72
7	3	76
8	7	74
9	9	328
10	8	100
11	8	216
12	7	98
13	7	224
14	9	128
15	8	232
16	7	56
Totals	112	2280

V_1C_o - Deltapine - Ceresan (check)

Rep	Plants	Total
1	6	172
2	7	272
3	5	108
4	7	224
5	5	120
6	5	160
7	5	100
8	3	56
9	6	192
10	6	306
11	8	196
12	9	182
13	7	164
14	7	218
15	7	158
16	6	134
Totals	99	2762

V_2C_o - Rowden - Ceresan (check)

Rep	Plants	Total
1	7	138
2	10	294
3	6	152
4	8	176
5	6	120
6	5	140
7	5	120
8	5	120
9	6	252
10	7	178
11	10	210
12	6	108
13	7	258
14	6	60
15	6	30
16	9	140
Totals	109	2496

V_1O_o - Deltapine - Omadine (check)

Rep	Plants	Total
1	9	376
2	5	220
3	10	500
4	4	128
5	6	320
6	8	290
7	5	140
8	6	208
9	6	126
10	5	140
11	8	196
12	7	178
13	7	204
14	2	44
15	8	210
16	6	54
Totals	102	3334

V_2O_o - Rowden - Omadine (check)

Rep	Plants	Total
1	7	244
2	9	188
3	6	172
4	7	304
5	6	192
6	6	192
7	7	284
8	5	120
9	7	304
10	7	284
11	9	182
12	8	150
13	7	158
14	9	78
15	8	54
16	8	146
Totals	116	3052

V_1S_o - Deltapine - Streptomycin
(check)

Rep	Plants	Total
1	7	112
2	4	88
3	6	232
4	4	68
5	9	368
6	8	190
7	3	76
8	5	60
9	9	268
10	6	132
11	8	170
12	6	152
13	6	132
14	6	157
15	8	278
16	5	36
Totals	100	2519

V_2S_o - Rowden - Streptomycin
(check)

Rep	Plants	Total
1	7	178
2	10	168
3	9	268
4	4	68
5	6	80
6	7	184
7	5	274
8	8	136
9	7	384
10	6	72
11	9	84
12	7	144
13	7	98
14	7	102
15	9	112
16	9	78
Totals	117	2430